THE CENTRAL AFRICAN JOURNAL OF MEDICINE

Vol. 47, No. 7 July 2001

CONTENTS

Antibiogram of *Klebsiella pneumoniae* isolates from Buea, Cameroon

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Abstract

Objective: To determine the antibiotic susceptibility of *K. pneumoniae* isolates from Buea, Cameroon.
Design: A prospective study of *K. pneumoniae* isolates from clinical samples of nosocomial origin.
Setting: A laboratory based investigative study at the Biotechnology Centres of the Universities of Buea and Yaounde 1, Cameroon, and three Buea based hospitals. *K. pneumoniae* isolates were obtained from sputum, wound swabs and urine and screened for their antibiogram using standard procedures.
Results: Results on the antibiogram showed seven distinct antibiotypes distinguished by different susceptibilities to aminoglycosides (Spectinomycin and Gentamicin), Chloramphenicol and Augmentin. All the isolates shared multi-resistance to Amoxicillin and Trimethoprim. However, the isolates showed marked susceptibilities to Norfloxacin (90.01%), Cefuroxime (95.45%) and Ciprofloxacin (86.36%).
Conclusion: The study has revealed that *K. pneumoniae* isolates in the environment of Buea, Cameroon are multi-drug resistant. This finding is of clinical and epidemiological significance.

Cent Afr J Med 2001;47(7):173-6

Introduction

Klebsiella pneumoniae is an important human pathogen that has been associated in recent decades with nosocomial outbreaks.[1,2] They are important opportunistic pathogens, commonly isolated from urinary tract infections, surgical wounds, nosocomial pneumonia and bloodstream infections.[3,4] These organisms are also an important source of transferable antibiotic resistance, and several outbreaks caused by multiple resistant *K. pneumoniae*, especially the extended-spectrum ß-lactamase-producing (ESBL) strains of the types TEM and SHV have been reported.[5-8]

Owing to their clinical significance, many methods exist for the epidemiological investigation of infections caused by this organism. In different parts of the world, biotyping, serotyping, antibiogram, plasmid profiles and more recent techniques like pulse field gel electrophoresis (PFGE) and random amplified polymorphic deoxyribonucleic acid (RAPD) analyses have been used in typing *Klebsiellae*.[9-12]

In the present study, we decided to use antibiogram as an epidemiological marker for *K. pneumoniae* isolates because there is a high frequency of drug abuse in the environment of Buea, Cameroon. The penicillins, Chloramphenicol, and to a lesser extent the aminoglycosides are relatively cheap, and therefore commonly available to the population; who tend to abuse them, heralding the emergence of resistance.[13]

K. pneumoniae are known to be resistant to a number of antibiotics, including extended-spectrum cephalosporins and aminoglycosides, because of the acquisition of plasmids which code for the production of ESBL and aminoglycoside-modifying enzymes.[10,14] However, it has been widely acclaimed that the susceptibility of pathogens to antibiotics varies with time and geographical location.[15,16] Since they account for a significant proportion of nosocomial infections and the tendency of nosocomial pathogens to develop or acquire new antibiotic resistance traits poses a great problem in their treatment and control,[1-4,10,17] it becomes necessary, therefore, to study the susceptibility patterns of *K. pneumoniae* isolates to some commonly used and relatively reserved antibiotics in Cameroon in order to update our knowledge on the use of these antimicrobial agents in the management of *K. pneumoniae* infections.

In Buea, Cameroon, there appears to be no report on the antibiogram and epidemiology of *K. pneumoniae* infections. In fact, we are not aware of any. It is against this background that the antibiogram of *K. pneumoniae* isolates was determined in an attempt to provide baseline data for clinical management and epidemiological surveys.

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Materials and Methods

Setting and Patients.

During a period of five months; April to August 2000, a total of 105 specimens comprising sputum, wound swabs, and urine were collected from hospitalised patients at the following medical centres in Buea, Cameroon; Buea District Hospital, Mount Mary Hospital and the Military Hospital. The patients included both males and females of all age groups. Infection due to *K. pneumoniae* was considered nosocomial if it appeared within 48 hours after admission according to the criteria of the Centers for Disease Control and Prevention.[18]

Bacterial Isolates.

Specimens (sputum, wound swabs and urine) were inoculated onto Eosin methylene blue agar (EMB) and MacConkey agar plates (MCA). Urine specimens were in addition inoculated onto cystein lactose electrolyte deficient (CLED) agar plates. The inoculated plates were incubated at 37°C for 24 hours, after which characteristic colonies were observed. Suspicious colonies were then sub-cultured on fresh EMB, CLED or MCA plates to obtain pure cultures[19] for further identification. Isolates were identified *as K. pneumoniae* by subjecting them to the API 20E (BioMerieux SA, France) screening Kit according to the manufacturer's instructions.

Antibiotic Susceptibility Testing.

The Kirby-Bauer disk diffusion test, which conforms to the recommended standard of the National Committee for Clinical Laboratory Standards (NCCLS),[20] was used as previously described.[21] Briefly, a small inoculum of each pure bacterial isolate was emulsified in 3ml sterile normal saline in Bijou bottles and the density compared to a barium chloride standard (0.5McFarland). A sterile cotton swab was dipped into the standardized solution of bacterial cultures and used to evenly inoculate Muller-Hinton plates (Biotec, England) and allowed to dry. Thereafter, antibiotic disks with the following drug contents — Amoxicillin (10μg); Gentamicin (10μg); Cefuroxime (30μg); Augmentin (30μg); Spectinomycin (30μg); Ofloxacin (5μg); Chloramphenicol (10μg); Ciprofloxacin (5μg); Norfloxacin (10μg) and Trimethoprim (5μg), (Oxoid, England) were placed on the plates, spacing them well to prevent the overlapping of inhibition zones. The plates were incubated at 37°C for 24 hours and the diameters were then compared with recorded diameters of the control organism, *E. coli* ATCC 25922 to determine susceptibility or resistance.[8,16,22]

Results

Antimicrobial Susceptibility Patterns.

Table I shows the antimicrobial susceptibility results exhibited by *K. pneumoniae* isolates. Of the 22 isolates tested, over 85% were sensitive to the fluoroquinolones (Norfloxacin, Ciprofloxacin, Ofloxacin) and the Cephalosporin, Cefuroxime. Most strains showed varied susceptibilities to the Aminoglycosides (Gentamicin and Spectinomycin), Augmentin and Chloramphenicol. However, all isolates showed total resistance (100%) to Amoxicillin and Trimethoprim. *K. pneumoniae* isolates were identified as belonging to seven different antibiotypes (Table II) distinguished by different susceptibilities to Aminoglycosides, Chloramphenicol and Augmentin. Most of the isolates (77.3%) were resistant to three or more antibiotics. The predominant antibiotype (Amx^R, Tmp^R) constituted nine (40.91%) of the isolates while the least patterns (Amx^R, Tmp^R Aug^R) and (Amx^R, Tmp^R, Chl^R Aug^R, Gen^R, Spc^R) were exhibited by one (4.54%) isolate respectively.

*Table I: Antibiotic sensitivity results of **K. pneumoniae** strains isolated from various clinical specimens.*

Antibiotics	Susceptible (%)	Resistant (%)
Trimethoprim (5μg)	0 (0%)	22 (100%)
Norfloxacin (10μg)	20 (90.01%)	2 (9.09%)
Ciprofloxacin (5μg)	19 (86.36%)	3 (13.64%)
Gentamicin (10μg)	17 (77.27%)	5 (22.73%)
Cefuroxime (30μg)	21 (95.45%)	1 (4.54%)
Augmentin (30μg)	14 (63.64%)	8 (36.36%)
Amoxicillin (10μg)	0	22 (100%)
Ofloxacin (5μg)	21 (95.45%)	1 (4.54%)
Chloramphenicol (10μg)	13 (59.09%)	9 (40.91%)
Spectinomicin (30μg)	18 (81.82%)	4 (18.18%)

*Table II: Antibiotypes of **K. pneumoniae** isolates.*

Antibiotype[a]	Number of strains with antibiotype	% with antibiotype
Amx^R, Tmp^R	9	40.91%
Amx^R, Tmp^R, Spc^R	3	13.64%
Amx^R, Tmp^R, Chl^R	2	9.09%
Amx^R, Tmp^R, Chl^R, Aug^R	2	9.09%
Amx^R, Tmp^R, Gen^R, Chl^R, Aug^R	4	18.18%
Amx^R, Tmp^R, Chl^R, Aug^R, Gen^R, Spc^R	1	4.54%
Amx^R, Tmp^R, Aug^R	1	4.54%

[a]Abreviations: Amx — Amoxicillin; Tmp — Trimethoprim; spc — Spectinomicin; Chl- Cloramphenicol; Aug — Augmntin; Gen — Gentamicin. [R] Resistance.

Discussion

K. pneumoniae accounts for a substantial degree of nosocomial infections[1,2] and the increasing tendency for nosocomial pathogens to acquire new antibiotic resistance traits poses a problem in chemotherapy[10,17,23] revealing the need for an updated antibiotic susceptibility pattern for the effective management of infections caused by these organisms. This study reports on the antibiogram of *K. pneumoniae*. Results revealed that *K. pneumoniae* isolates showed marked susceptibilities to Ciprofloxacin, Norfloxacin, Ofloxacin, Cefuroxime and Gentamicin (Table I). The extreme susceptibility (95.45%) to the cephalosporin, Cefuroxime; the quinolones, Ofloxacin (95.45%), Norfloxacin (90.10%) and Ciprofloxacin

(86.36%); the aminoglycosides, Spectinomycin (81.82%) and Gentamicin (77.27%) is at variance with those of other investigators.[1,2,4,10,24,25]

Though one isolate in this study showed[4] low levels of susceptibilities to these drugs, the others documented extreme resistance to them, which they attributed to the production of ESBL. We speculate that the observed unusual difference could be related to the limited use of these drugs in Cameroon due to their high costs, therefore limiting the selection of resistant mutants. This could be further supported by the fact that previous studies have reported a wide distribution of these enzymes with the TEM-3 more prevalent in Europe, TEM-10, TEM-12 and TEM-26 in the USA, SHV-2 and SHV-5 world wide except sub-Saharan Africa.[26]

Until recently when it was reported in South Africa,[22] these enzymes had been isolated from the Saharan African countries of Tunisia and Egypt.[27] On the other hand, we are constrained to speculate that the non determination of ESBL production in our strains may be a contributing factor to this unusual susceptibility, especially to the cephalosporins, because production of ß-lactamases is known to cause hidden resistance to the expanded-spectrum cephalosporins.[28] Lower levels of susceptibility to Augmentin (63.64%) and Chloramphenicol (59.09%) were also observed corroborating a previous finding,[4] even though a high degree of susceptibility (>80%) of the *Klebsiellae* to Chloramphenicol and Augmentin had been documented.[29] This could be linked to the fact that Chloramphenicol, commonly used in our environment to treat typhoid fever, is highly abused[13] thus putting a selective pressure on the drug, hence the emergence of resistance to it .

Results of this study revealed seven distinct resistance patterns (antibiotypes), of which the most prevalent exhibited resistance to Amoxicillin and Trimethoprim (Amx^R, Tmp^R) accounting for 40.91% of the isolates (Table II). This is also in agreement with the findings of Livrelli *et al.*[4] The least patterns (Amx^R, Tmp, Chl^R, Aug^R, Gen^R, Spc^R) and (Amx^R, Tmp^R, Aug^R) were exhibited by one (4.55%) isolate respectively. All the antibiotypes shared resistance to Amoxicillin and Trimethoprim. This suggests that these drugs may not be useful in the treatment of *K. pneumoniae* infections in this area.

An interesting feature of this study is that all *K. pneumoniae* isolates were resistant to two or more antibiotics. The multiple resistance to these drugs could be attributed to the fact that these antibiotics are highly abused due to constant and indiscriminate usage.[13] Also, *Klebsiellae* are known to be an important source of transferable antibiotic resistance among Gram-negative bacilli.[5-8,30] Consequently, the occurrence of multiply resistant *Klebsiella* strains might pose a potential problem in the treatment of infections in the study area.

With the use of an antibiogram, this study has provided baseline data for epidemiological research on *K. pneumoniae* infections in the environment of Buea, Cameroon. We, however, suggest the need to adopt discriminatory molecular typing methods such as RAPD and PFGE to complement the typing of *K. pneumoniae* isolates in our environment.

Acknowledgements

We are grateful to the University of Buea for providing equipment and some reagents used in this study. We also appreciate the cooperation received from the management of the Buea District Hospital, Mount Mary and the Military Hospitals, Buea where samples were collected.

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